REMARKS

The 35 U.S.C. §103(a) Rejection

Claims 1-3, 6 and 24-29 stand rejected under 35 U.S.C. §103(a) as being unpatentable over **Toida** et al. (*Infect. Immunity* 65:909, 1997) in view of **Rappuoli** et al. (*Immunol. Today* 20:493, 1999), and further in view of **Schodel** et al. (*Infect. Immunity*, 57:1347, 1989; *Vaccine* 8:569, 1990) and **Connell** et al. (*Immuol. Lett.* 62:117, 1998; *Infect. Immunity* 60:1653, 1992). The rejection is respectfully traversed.

Claim 1 is drawn to a method of inducing cellular immune response by a fusion protein comprising an antigen fused to the A2 and B subunits of a type II heat-labile enterotoxin. In contrast, **Toida** et al. teach a method of inducing immune responses using a chimeric immunogen comprising an antigen fused to the A2 and B subunits of cholera toxin. **Rappuoli** et al. teach the structure and mucosal adjuvanticity of cholera and *E. coli* heat labile enterotoxin. **Rappuoli** et al., however, do not distinguish type II heat-labile enterotoxin from type I heat-labile enterotoxin. **Schodel** et al. teach inducing immune responses using an attenuated *Salmonella* expressing a fusion protein consists of an antigen fused to the B

subunit of *E. coli* heat labile enterotoxin. **Schodel** et al. also do not distinguish type II heat-labile enterotoxin from type I heat-labile enterotoxin.

Toida et al. only teach cholera toxin. **Rappuoli** et al. and **Schodel** et al. teach heat labile enterotoxin, but the teachings do not distinguish between type I and type II heat labile enterotoxins. **Rappuoli** et al. teach cholera toxin and heat labile enterotoxin have high homology (80% identity) in their primary structure (page 493, right column, first two sentences of second paragraph). However, this statement is only half true. Although the B chain of cholera toxin and type I heat labile enterotoxin have 80% identity, the B chain of cholera toxin and type I heat labile enterotoxin have little or no homology with the B chain of type II heat labile enterotoxin (**Connell** et al., 1998, page 118, left column, lines 14-17). The differences in amino acid sequences are significant in that antibodies directed against type I and type II heat labile enterotoxins will not cross-react with each other (**Connell** et al., 1998, page 118, left column, lines 18-27). Hence, there is significant structural difference between type I and type II heat labile enterotoxins. Apparently, the heat labile enterotoxin in **Rappuoli** et al. refers only to type I, not type II, enterotoxin. Applicants submit that the

lack of distinction between these two types of enterotoxins in **Rappuoli** et al. (similarly in **Schodel** et al.) is a reflection of confusion and/or lack of understanding about type II heat labile enterotoxins in the prior art.

The Examiner argues that the gap in teaching on type II heat-labile enterotoxin can be filled by **Connell** et al. The Examiner contends that **Connell** et al. teach type I and type II heat labile enterotoxins can be used as mucosal adjuvants; that type I and type II heat labile enterotoxins have conserved structural features that permit their A and B chains to form hybrid holotoxin; hence, it is well known in the art that the structural difference of the B subunit does little toward the adjuvanticity of type I and type II heat labile enterotoxins. Applicants respectfully disagree.

The issue is whether the prior art teaches there are insignificant structural and functional differences between type I and type II heat labile enterotoxins. Applicants submit that the B subunits of type I and type II heat labile enterotoxins have significant structural differences that would lead one of ordinary skill in the art to expect functional differences between the two types of enterotoxins.

First of all, Applicants submit that the Examiner has misinterpreted **Connell** et al. (1992) to contend that there are insignificant structural differences between the B subunits of type I and type II heat labile enterotoxins. Although subunits of type I and type II heat labile enterotoxins can form heterologous hybrids, the resulting hybrids exhibited drastically reduced biological activities (up to 1000-fold, see **Connell** et al., 1992, Table 2, compare the type I (subunit A)/type II (subunit B) hybrids to their wild-type homologous combinations). Moreover, the type I (subunit A)/type II (subunit B) hybrids underwent significant conformational changes so that they were not recognized by anti-subunit A antibody (**Connell** et al., 1992, Table 2, footnote c) or anti-subunit B antibody (**Connell** et al., 1992, Table 3, footnote c). In view of these significant reduction of biological activities and conformational changes, one of ordinary skill in the art would readily recognize that there is significant differences between the B subunits of type I and type II enterotoxins.

Connell et al. (1992) stated that "type I and type II enterotoxins have conserved structural features that permit their A and B polypeptides to form hybrid holotoxins, although the B polypeptides of the type I and type II enterotoxins have very little

amino acid sequence homology." This statement, however, does not mean there are no structural differences between the B subunits of the two enterotoxins. What the author (who is also the co-inventor of the present application) meant was the B subunits of the type I and type II enterotoxins are not related evolutionarily, but they may have evolved some analogous functions (such as delivering the A subunits into cells) by a process of convergent evolution. The data in Tables 2 and 3 as described above clearly indicate that there are significant structural (and possibly functional) differences between the B subunits of the two enterotoxins.

The B subunit of heat labile enterotoxin mediates binding to cell surface receptor, thereby directing the toxin to the cells (**Rappuoli** et al., Figure 1). As discussed above, there is little or no sequence identity between the B subunits of type I and type II heat labile enterotoxins. Consequently, the two types of enterotoxins are directed to different cells by the different B subunits. Cholera toxin and type I heat labile enterotoxin bind preferentially to ganglioside GM1, whereas type II heat labile enterotoxin binds preferentially to gangliosides GD1b or GD1a (**Connell** et al., 1998, page 1653, last sentence on left column to first sentence on right column). "The different receptor binding activities of heat labile enterotoxin and

cholera toxin might be significant for the qualitatively different immunological properties that are exhibited by the [toxins]" (**Rappuoli** et al., page 495, left column, last sentence of first paragraph). The functional importance of receptor binding mediated by the B subunit is further supported by the finding that shows mutation at the B subunit of heat labile enterotoxin abolishing receptor binding also renders the toxin non-immunogenic at mucosal surface (**Rappuoli** et al., page 496, right column, first paragraph). Non-receptor binding B subunit mutants also lose other immune-modulating activities such as inducing of apoptosis (**Rappuoli** et al., page 496, right column, first paragraph). Hence, in view of the structural differences between type I and type II heat labile enterotoxin B subunit and the functional importance of the B subunit, one of ordinary skill in the art would reasonably expect different immunological functions for the two types of enterotoxins.

The Examiner also contends that **Connell** et al. (1998) teach type I and type II heat labile enterotoxins can be used as mucosal adjuvants, indicating that there is functional similarity between the two types of enterotoxins. Applicants submit that this teaching may not be relevant to the present invention. **Rappuoli** et al. teach that adjuvant activity was retained in recombinant enterotoxin that only

contained the A subunit (page 497, left column, second paragraph), whereas recombinant enterotoxin that only contained the B subunit was poor adjuvant (page 498, right column, last paragraph). The present invention is drawn to an immunogen comprising the A2 (the carboxyl terminal of A subunit) and B subunits of a type II heat-labile enterotoxin. In view of the teaching of **Rappuoli** et al., one of ordinary skill in the art would reasonably conclude that the A2B complex of the present invention would have minimal, if there is any, adjuvant activity.

In summary, the prior art references cited by the Examiner only clearly teach the immunological properties of type I heat labile enterotoxin and substantial structural differences between the B subunits of type I and type II enterotoxins. The cited prior art does not teach or suggest (neither would one of ordinary skill in the art expect) there is any functional similarity between the two enterotoxins in spite of the structural differences. One of ordinary skill in the art could not reasonably deduce without empirical experimentation immunomodulatory functions for the type II enterotoxin of the instant invention based on the published properties of type I enterotoxin. Hence, the prior art does not

provide one of ordinary skill in the art with sufficient guidance to arrive at the invention of claims 1-3 and 6.

Claims 24-29 are drawn to methods of increasing Th1 response and cell-mediated immunity by a fusion protein comprising an antigen fused to the A2 and B subunits of a type II heat-labile enterotoxin. The Examiner contends that **Rappuoli** et al. teach heat labile enterotoxin could activate both Th1 and Th2 cells. However, as discussed above, the heat labile enterotoxin in **Rappuoli** et al. refers only to type I, not type II, enterotoxin. Applicants submit that the prior art references cited by the Examiner as a whole do not teach or suggest any functional similarity between type I and type II enterotoxins with regard to their abilities to induce Th1 response and cell-mediated immunity. Indeed, in view of the significant structural differences taught by the cited prior art, one of ordinary skill in the art would reasonably expect differences in the induction of Th1 responses by the two enterotoxins. Hence, Applicants submit that the cited prior art does not provide one of ordinary skill in the art with sufficient guidance to arrive at the invention of claims 24-29.

The Examiner contends that the instant situation is amenable to the type of analysis set forth in In re Kerkhoven, 205 USPQ 1069

(CCPA 1980) wherein the court held that it is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose. Applicants respectfully disagree. Applicants submit that the prior art has clearly taught significant structural differences between type I and type II enterotoxins, but the prior art does not teach or suggest the two enterotoxins would have the same or similar immunostimulatory functions in spite of their differences. Hence, Applicants submit that the instant invention is not amenable to the type of analysis set forth in In re Kerkhoven.

In view of the above remarks, the combined teaching of the cited references does not provide a person having ordinary skill in this art with the requisite expectation of successfully producing Applicants' claimed methods. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 1-8 and 24-29 under 35 U.S.C. §103(a) be withdrawn.

Claims 1-3, 6 and 24-29 stand rejected under 35 U.S.C. §103(a) as being unpatentable over **Russell** et al. (US patent

6,030,624) in view of **Rappuoli** et al. (*Immunol. Today* 20:493, 1999), and further in view of **Schodel** et al. (*Infect. Immunity*, 57:1347, 1989; *Vaccine* 8:569, 1990) and **Connell** et al. (*Immuol. Lett.* 62:117, 1998; *Infect. Immunity* 60:1653, 1992). The rejection is respectfully traversed.

Russell et al. teach a method of inducing immune responses using a chimeric immunogen comprising an antigen fused to the subunits of cholera toxin. The other cited references have been discussed above. **Rappuoli** et al., **Schodel** et al. and **Connell** et al. have been described above.

The Examiner rejects the claims on the same basis as that based on **Toida** et al., **Rappuoli** et al., **Schodel** et al. and **Connell** et al. Therefore, the above discussion applies here also. The issue is whether the prior art teaches there are insignificant structural and functional differences between type I and type II heat labile enterotoxins so that one of ordinary skill in the art would find it obvious to replace the cholera toxin in the immunogen of **Russell** et al. (or **Toida** et al.) with components of type II enterotoxin and expect to come up with the same or similar immunological properties. As discussed above, the cited prior art clearly teaches significant structural differences between type I and type II

enterotoxins, but the prior art does not provide any teaching or suggestion that would reasonably enable one of ordinary skill in the art to expect the same or similar immunological properties between cholera toxin/type I enterotoxin and type II enterotoxin. Even though one skilled in the art might find it obvious to try various combinations of the elements culled from these references; however, "obvious to try" is not the standard of 35 U.S.C. §103. Applicants reassert that obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention absent some teaching or suggestion supporting the combination. Applicants aver that no such teaching or suggestion may be gleaned from the references relied upon by the Examiner.

In view of the above remarks, Applicants submit that the combined teaching of the cited references does not render the instant invention obvious. The combined teaching only teaches a method of using cholera toxin or type I enterotoxin. The combined teaching does not teach or suggest induction of cellular immune responses by type II enterotoxins as claimed herein. Hence, the cited references do not provide a person having ordinary skill in this art with the requisite expectation of successfully producing Applicants' claimed methods. The invention as a whole is not *prima*

facie obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 1-3, 6 and 24-29 under 35 U.S.C. §103(a) be withdrawn.

This is intended to be a complete response to the Final Office Action mailed May 6, 2003. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

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Benjamin Aaron Adler, Ph.D., J.D.
Registration No. 35,423
Counsel for Applicant

ADLER & ASSOCIATES
8011 Candle Lane
Houston, Texas 77071
(713) 270-5391 (tel.)
(713) 270-5361 (facs.)
badler1@houston.rr.com

AMENDMENTS IN THE CLAIMS

1. (previously presented) A method of inducing an immune response by administration of a recombinant immunogen comprising a fusion protein of an antigen fused to the A2 and B subunits of a type II heat-labile enterotoxin, wherein said immune response is selected from the group consisting of development of antigen-specific T cells in the circulation and tissues, the development of cytotoxic T cells and immunological tolerance to the antigen sequence.

2. (original) The method of claim 1, wherein said antigen of interest is salivary binding protein (SBR) from *Streptococcus mutans* surface protein (Ag I/II).

3. (original) The method of claim 1, wherein said type II heat-labile enterotoxin is selected from the group consisting of *E. coli* heat-labile type IIa toxin and *E. coli* heat-labile type IIb toxin.

4-5. (canceled)

6. (original) The method of claim 1, wherein said immunogen is administered by a route selected from the group consisting of orally, intranasally, intrarectally, intravaginally, intramuscularly, transcutaneously and subcutaneously.

7-23. (canceled)

24. (previously presented) A method of increasing Th1 response and cell-mediated immunity by administration of a recombinant immunogen comprising a fusion protein of an antigen fused to the A2 and B subunits of a type II heat-labile enterotoxin.

25. (original) The method of claim 24, wherein said antigen of interest is salivary binding protein (SBR) from *Streptococcus mutans* surface protein (Ag I/II).

26. (original) The method of claim 24, wherein said immunogen is administered by a route selected from the group consisting of orally, intranasally, intrarectally, intravaginally, intramuscularly, transcutaneously and subcutaneously.

27. (previously presented) A method of increasing Th1 response and cell-mediated immunity by administration of a recombinant immunogen comprising a fusion protein of an antigen fused to the A2 and B subunits of a *E. coli* heat-labile type IIa or type IIb toxin.

28. (original) The method of claim 27, wherein said antigen of interest is salivary binding protein (SBR) from *Streptococcus mutans* surface protein (Ag I/II).

29. (original) The method of claim 27, wherein said immunogen is administered by a route selected from the group consisting of orally, intranasally, intrarectally, intravaginally, intramuscularly, transcutaneously and subcutaneously.